

Claims:

1. A method for the production of ascorbic acid or an ascorbic acid stereoisomer in a yeast comprising the steps of:

5           a) obtaining a yeast capable of utilizing KLG to produce ascorbic acid or an ascorbic acid stereoisomer; and

          b) culturing the yeast in the presence of a carbon source under conditions suitable for the production of ascorbic acid or an ascorbic acid stereoisomer.

10           2. The method of Claim 1 further comprising the step of recovering said ascorbic acid.

          3. The method of Claim 1 wherein said carbon source is a six carbon sugar acid.

15           4. The method of Claim 3 wherein said six carbon sugar acid includes 2-keto-L-gulonic acid, idonic acid, gluconic acid, 6-phosphogluconate, 2-keto-D-gluconic acid, 5-keto-D-gluconic acid, 2-ketogluconate-6-phosphate, 2, 5-diketo-L-gluconic acid, 2,3-L-diketogulonic acid, dehydroascorbic acid, erythroascorbic acid and D-mannonic acid

20           5. The method of Claim 1 wherein said carbon source is a six carbon sugar and said yeast comprises either or both of a) a heterologous nucleic acid encoding an oxidative enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast and b) a heterologous nucleic acid encoding a reducing enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast.

25           6. The method of Claim 5 wherein said six carbon sugar includes glucose, gulose, idose, galactose, mannose, sorbose and fructose.

7. The method of Claim 5 wherein said oxidative enzyme has a dehydrogenase activity.

8. The method of Claim 7 wherein said dehydrogenase includes a glucose dehydrogenase activity, a gluconic acid dehydrogenase activity, a 2-keto-D-gluconic acid dehydrogenase activity, a galactose dehydrogenase activity, an L-sorbose activity, a D-sorbitol dehydrogenase activity, L-sorbosone dehydrogenase activity, L-idonic acid oxidase and L-gulonic acid oxidase.

10 9. The method of Claim 5 wherein said reducing enzyme is a reductase activity.

10. The method of Claim 9 wherein said reductase activity includes 2,5 DGK reductase activity, 2,5 DKG reductase activity, 2,3-DKG reductase, 5-keto reductase, 2-keto reductase and 2 ketogulonate reductase.

15 11. The method of Claim 1 wherein said carbon source is glucose and the yeast comprises heterologous nucleic acid encoding at least one of (a) a glucose dehydrogenase (GDH); (b) a gluconic acid dehydrogenase (GADH); (c) a 2-keto-D-gluconic acid dehydrogenase (2-KDGDH); and (d) a 2,5-diketo-D-gluconic acid reductase (2,5-DGKR) provided that if the yeast comprises heterologous nucleic acid for less than all of (a) - (d), then the yeast comprises endogenous nucleic acid such that the yeast comprises nucleic acid for each of (a) - (d) and is capable of converting glucose to ASA via the intermediate KLG.

20 25 12. The method of Claim 1 wherein the yeast is a member of the Imperfect yeast group.

13. The method of Claim 12 wherein the yeast is a member of the family Cryptococcaceae.

30 14. The method of Claim 13 wherein the yeast includes *Candida* and *Cryptococcus*.

15. The method of Claim 14 wherein the yeast is *Candida blankii*.

16. The method of Claim 14 wherein the yeast is *Cryptococcus dimennae*.

5 17. The method of Claim 1 wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* and said carbon source comprises glucose, wherein said yeast comprises a heterologous glucose dehydrogenase activity and a 2,5-DKGreductase activity.

10 18. The method of Claim 1 wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* and said carbon source comprises D-sorbitol, L-sorbose or L-sorbosone, wherein said yeast comprises at least one of an L-sorbose activity, a D-sorbitol dehydrogenase activity, an L-sorbosone dehydrogenase activity, and a galactose dehydrogenase activity.

15 19. The method of Claim 1 wherein said ascorbic acid stereoisomer includes D-ascorbic acid, D-araboascorbic acid and L-araboascorbic acid.

20 20. A recombinant yeast capable of utilizing KLG to produce ascorbic acid or an ascorbic acid stereoisomer comprising either one or both of a) a heterologous nucleic acid encoding an oxidative enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast and b) a heterologous nucleic acid encoding a reducing enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast.

25 21. The yeast of Claim 20 wherein said oxidative enzyme is a dehydrogenase activity.

22. The yeast of Claim 21 wherein said dehydrogenase includes a glucose dehydrogenase activity, a gluconic acid dehydrogenase activity, a 2-keto-D-gluconic acid dehydrogenase activity, a galactose dehydrogenase activity, an L-

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sorbose activity, a D-sorbitol dehydrogenase activity, L-sorbosone dehydrogenase activity, L-idonic acid oxidase and L-gulonic acid oxidase.

5            23. The yeast of Claim 20 wherein said reducing enzyme is a reductase activity.

10            24. The yeast of Claim 23 wherein said reductase activity includes 2,5 DKG reductase activity, 2,5 DKG reductase activity, 2,3-DKG reductase, 5-keto reductase, 2-keto reductase and 2 ketogulonate reductase.

15            25. The yeast of Claim 20 wherein the yeast is a member of the Imperfect yeast group.

20            26. The yeast of Claim 25 wherein the yeast is a member of the family Cryptococcaceae.

25            27. The yeast of Claim 26 wherein the yeast includes *Candida* and *Cryptococcus*.

30            28. The yeast of Claim 27 wherein the yeast is *Candida blankii*.

35            29. The yeast of Claim 27 wherein the yeast is *Cryptococcus dimennae*.

40            30. The yeast of Claim 20 wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* and said carbon source comprises glucose, wherein said yeast comprises a heterologous glucose dehydrogenase activity and a 2,5-DKG reductase activity.

45            31. The yeast of Claim 20 wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* and said carbon source comprises D-sorbitol, L-sorbose or L-sorbosone, wherein said yeast comprises at least one of an L-sorbose activity, a D-sorbitol dehydrogenase activity, an L-sorbosone dehydrogenase activity, and a galactose dehydrogenase activity.

32. A method for producing a recombinant yeast capable of utilizing a six carbon sugar to produce ASA or an ASA stereoisomer comprising the steps of:

- a) obtaining a yeast capable of utilizing KLG to produce ASA or an ASA stereoisomer and
- 5 b) introducing at least either or both of a) a heterologous nucleic acid encoding an oxidative enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast and b) a heterologous nucleic acid encoding a reducing enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast.

10 33. The method of Claim 32 wherein said yeast is a member of the Imperfect yeast group.

15 34. The yeast of Claim 33 wherein the yeast is a member of the family Cryptococcaceae.

35. The yeast of Claim 34 wherein the yeast includes Candida and Cryptococcus.

20 36. The yeast of Claim 35 wherein the yeast is Candida blankii.

37. The yeast of Claim 35 wherein the yeast is Cryptococcus dimennae.

25 38. The yeast of Claim 32 wherein said yeast is Candida blankii or Cryptococcus dimennae and said carbon source comprises glucose, wherein said yeast comprises a heterologous glucose dehydrogenase activity and a 2,5-DKGreductase activity.

30 39. The yeast of Claim 32 wherein said yeast is Candida blankii or Cryptococcus dimennae and said carbon source comprises D-sorbitol, L-sorbose or L-sorbosone, wherein said yeast comprises at least one of an L-sorbose activity, a D-sorbitol dehydrogenase activity, an L-sorbosone dehydrogenase activity, and a galactose dehydrogenase activity.

40. A method for screening for yeast capable of producing ASA comprising the steps of obtaining yeast capable of growing on ascorbic acid or ascorbic acid stereoisomer, culturing said yeast in the presence of KLG under conditions suitable for the production of ascorbic acid or an ascorbic acid stereoisomer; and assaying said yeast culture for the production of ascorbic acid or an ascorbic acid stereoisomer.